

Figure S1. Disorder prediction for the hypervariable region in rat ENaC subunits. Residue-wise disorder was predicted using Disopred2 (<http://bioinf.cs.ucl.ac.uk/disopred/>). The ‘filter’ curve (continuous black line) represents the predicted probability of disorder for the corresponding amino acid. The horizontal dashed line represents the order/disorder threshold for the default false positive rate of 5%. The ‘output’ curve (dashed curve) represents the level of confidence in prediction of disorder for the corresponding amino acid. **A, B, C)** Disorder prediction for corresponding regions in α -, β - and γ -ENaC respectively.

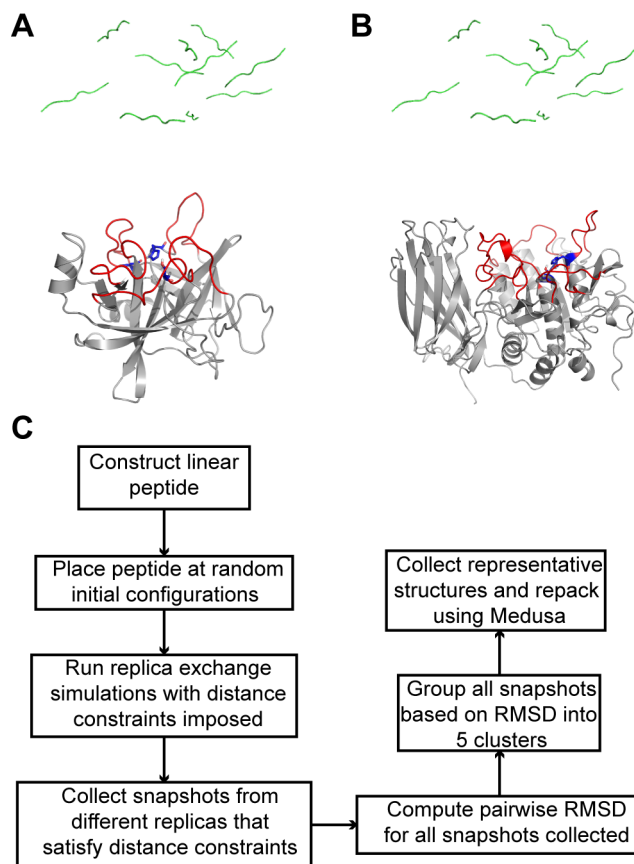


Figure S2. Simulation system and protocol. **A, B)** Starting conformations of CAP3 and furin respectively, with peptide Seq1 (colored green) placed at random positions at least 10Å away from the active site residues (colored blue and shown in stick representation). Portion of each protease shown in grey is maintained static during simulation while the loops shown in red are left flexible. Active site residues are static during the simulation. **C)** The steps outlined in the flowchart were followed for modeling the enzyme-substrate complexes. Eight replicas were used for modeling with a starting temperature of 0.5 and a step of 0.035.

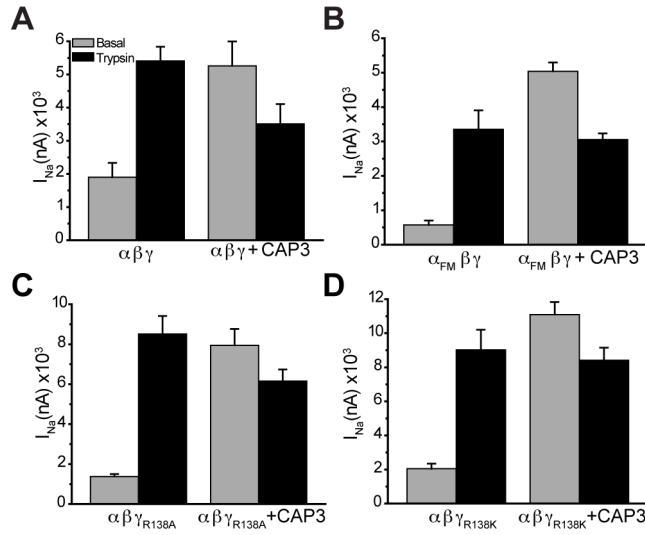


Figure S3. CAP3 coexpression stimulates ENaC containing mutant furin sites. A) Control experiment with wildtype ENaC and CAP3. **B)** Mutation of two α -ENaC furin sites (FM: R_{205,231}A) reduced basal I_{Na} , as expected, but did not prevent full proteolytic stimulation of I_{Na} by co-expressed CAP3. **C, D)** Two mutations of the furin site identified in γ -ENaC had no effect on CAP3 stimulation of ENaC. Methods, replications and analysis similar as described for Figure 3.

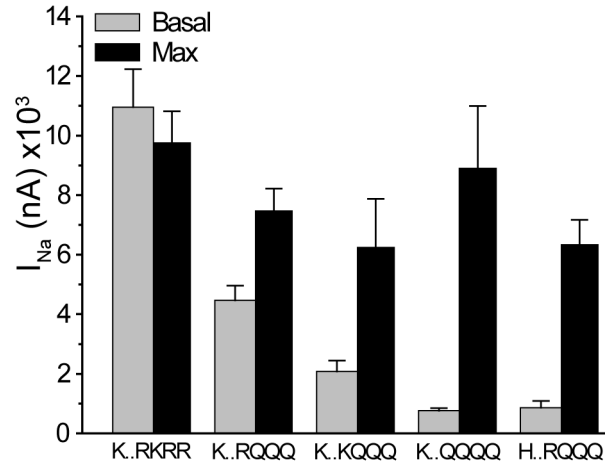


Figure S4. Residue 135R in γ -ENaC can form a CAP3-sensitive cleavage site with 132K. Oocytes were injected with 0.3 ng each of WT α - and α -subunits and γ -subunits bearing the 132..135-138 residues indicated above. CAP3 was co-expressed in each group. I_{Na} was recorded after 24 hr expression (grey bars). Following 5 min exposure to 20 μ g/ml trypsin or 2 μ g/ml hNE, I_{Na} was recorded again (black bars). The proteolytic stimulation of I_{Na} by co-expressed CAP3 was maximal with WT γ -ENaC. CAP3 partially stimulated 132KESRQQQ and 132KESKQQQ to a lesser degree. This stimulation was ablated by a non-basic residue at 135 or by Histidine at residue 132.

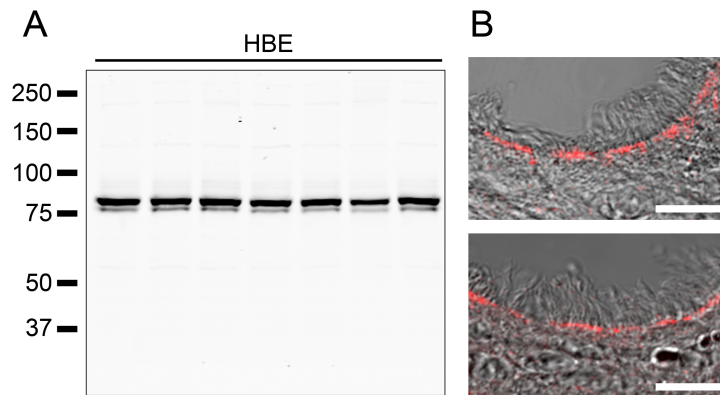


Figure S5. Matriptase is expressed in well-differentiated primary human bronchial epithelial (HBE) cultures. Matriptase was detected by Western blotting (A) and by immunofluorescence microscopy (B) using purified rabbit anti-Matriptase antibody (Bethyl Laboratories Inc., Cat. No. A300-221A). 50 μ g protein lysates of HBE cultures from different donors were loaded for analysis by Western blot. Confocal immunofluorescence microscopy was performed on frozen culture sections of primary HBE cells using goat-anti rabbit Alexa Fluor 568 IgG conjugate. Overlay with DIC shows expression in subapical compartments of ciliated HBE cells. Images were obtained using a Zeiss 510 Meta Laser Scanning Confocal Microscope. Bar = 10 μ m.